IB/2004/050736

Sertifikaat

REPUBLIEK VAN SUID AFRIKA

PATENT KANTOOR
DEPARTEMENT VAN HANDEL
EN NYWERHEID

Certificate

REPUBLIC OF SOUTH AFRICA

PATENT OFFICE
DEPARTMENT OF TRADE AND
INDUSTRY

Hiermee word gesertifiseer dat
This is to certify that

1) South African Provisional Patent Application No. **2003/1344** accompanied by a Provisional Specification was originally filed at the South African Patent Office on **19 February 2003**, in the name of **GAMMATRON (PTY) LIMITED** in respect of an invention entitled: **" Preparation of an Osteoinductive Agent ".**

2) On 12 January 2004, the application was postdated to **19 May 2003**. By virtue of such postdating, the effective filing date of the application is **19 May 2003.**

3) On 18 May 2004 an assignment of South African Patent application **No. 2003/1344** from **GAMMATRON (PTY) LIMITED** to **DU PLESSIS, Tjaart Andries** and **DE VILLIERS, Malan** was recorded at the South African Patent Office.

4) The photocopy attached hereto is a true copy of the provisional specification and drawing filed with South African Patent Application No. **2003/1344.**

Geteken te **PRETORIA** in die Republiek van Suid-Afrika, hierdie **8th** dag van **July 2004**

in the Republic of South Africa, this day of

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Registrar of Patents

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SUBMITTED OR TRANSMITTED IN
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REPUBLIC OF SOUTH AFRICA

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PATENTS ACT,

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OFFICIAL APPLICATION NO.	LODGING DATE : PROVISIONAL	ACCEPTANCE DATE
21 2003/1344	22 ~~10 February 2003~~ Backdated 19.05.2003	43
INTERNATIONAL CLASSIFICATION	**LODGING DATE : COMPLETE**	**GRANTED DATE**
51	23	

FULL NAME(S) OF APPLICANT(S) / PATENTEE(S)

71 GAMMATRON (PTY) LTD

APPLICANTS SUBSTITUTED : DATE REGISTERED

71 DU PLESSIS, Tjaart Andries, DE WAAL, Malan — APPLICANTS SUBSTITUTED 05-04.

ASSIGNEE(S) DATE REGISTERED

71

FULL NAME(S) OF INVENTOR(S)

72 DU PLESSIS, Tjaart Andries

PRIORITY CLAIMED	COUNTRY	NUMBER	DATE
N.B. Use international abbreviation for country. (See Schedule 4)	33	31	32

TITLE OF INVENTION

54 PREPARATION OF AN OSTEOINDUCTIVE AGENT

ADDRESS OF APPLICANT(S) / PATENTEE(S)

298 Stokkiesdraai
Erasmusrand
Pretoria
South Africa

ADDRESS FOR SERVICE

74 D M Kisch Inc, 54 Wierda Road West, Wierda Valley, SANDTON — REF P26152ZA00

PATENT OF ADDITION NO.	DATE OF ANY CHANGE
61	
FRESH APPLICATION BASED ON	**DATE OF ANY CHANGE**

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

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PBHR 229

APPLICATION FOR A PATENT AND ACKNOWLEDGEMENT OF RECEIPT
(Section 30 (1) - Regulation 22)
The grant of a patent is hereby requested by the undermentioned applicant on the basis of the present application filed in duplicate.

OFFICIAL APPLICATION NO.	
21	01 2003/1344

DMK REFERENCE
P26152ZA00

FULL NAME(S) OF APPLICANT(S)

71 GAMMATRON (PTY) LTD

ADDRESS(ES) OF APPLICANT(S)

298 Stokkiesdraai
Erasmusrand
Pretoria
South Africa

TITLE OF INVENTION

54 PREPARATION OF AN OSTEOINDUCTIVE AGENT

THE APPLICANT CLAIMS PRIORITY AS SET OUT ON THE ACCOMPANING FORM P2
The earliest priority claimed is

THIS APPLICATION IS FOR A PATENT OF ADDITION TO PATENT APPLICATION NO. 21 01

THIS APPLICATION IS FRESH APPLICATION IN TERMS OF SECTION 37 AND BASED ON APPLICATION NO. 21 01

THIS APPLICATION IS ACCOMPANIED BY :

X	1a	A single copy of a provisional specification of 15 pages.
	1b	Two copies of a complete specification of pages.
	2a	Informal drawings of sheets.
X	2b	Formal drawings of 1 sheets.
	3	Publication particulars and abstract (form P8 in duplicate).
	4	A copy of figure of the drawings for the abstract.
X	5	Assignment of invention (from the inventors) or other evidence of title.
	6	Certfied priority document(s).
	7	Translation of priority document(s).
	8	Assignment of priority rights.
	9	A copy of form P2 and a specification of S.A. Patent Application. 21 01
X	10	A declaration and power of attorney on form P3.
	11	Request for ante-dating on form P4.
	12	Request for classification on form P9.
	13a	Request for delay of acceptance on form P4.
	13b	

DATED 19 February 2003

Patent Attorney for Applicant(s)

REGISTRAR OF PATENTS, DESIGNS, TRADE MARKS AND COPYRIGHT
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REGISTRATEUR VAN PATENTE, MODELLE, HANDELSMERKE EN OUTEURSREG

ADDRESS FOR SERVICE

74 D M Kisch Inc
Inanda Greens Business Park
54 Wierda Road West
Wierda Valley
SANDTON

OFFICIAL DATE STAMP
19 FEB 2003

REGISTRAR OF PATENTS

The duplicate will be returned to the applicant's address for service as proof of lodging but is not valid unless endorsed with official stamp

D.M. KISCH INC., Sandton

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

Form P 3

DECLARATION AND POWER OF ATTORNEY
(Section 30 - Regulation 8, 22(i)(c) and 33)

PATENT APPLICATION NO.	LODGING DATE	D M KISCH'S REFERENCE
21 2003/1344	22 19.02.2003	AvR P26152ZA00

FULL NAME(S) OF APPLICANT(S)
71 GAMMATRON (PTY) LTD

FULL NAME(S) OF INVENTOR(S)
72 DU PLESSIS, Tjaart Andries

EARLIEST PRIORITY CLAIMED	COUNTRY	NUMBER	DATE
NOTE: The country must be indicated by its International abbreviation - see Schedule 4 of the Regulations	33 -	31 -	32 -

TITLE OF INVENTION
54 PREPARATION OF AN OSTEOINDUCTIVE AGENT

I **Tjaart Andries du Plessis**

hereby declare that --

1. ~~I am the applicant mentioned above;~~
2. I have been authorised by the applicant to make this declaration and have knowledge of the facts herein stated in the capacity of **DIRECTOR** of the applicant;
3. the inventor of the abovementioned invention is the person named above and the applicant has acquired the right to apply by virtue of an *assignment from the inventor;*
4. to the best of my knowledge and belief, if a patent is granted on the application, there will be no lawful ground for the revocation of the patent;
5. ~~this is a convention application and the earliest application from which priority is claimed as set out above is the first application in a convention country in respect of the invention claimed in any of the claims; and~~
6. the directors and qualified staff of the firm of **D M KISCH INC**, patent attorneys, are authorised, jointly and severally with powers of substitution and revocation, to represent the applicant in this application and to be the address for service of the applicant while the application is pending and after a patent has been granted on the application.

SIGNED AT PRETORIA THIS 18th DAY OF FEBRUARY 2003.

.....
T A DU PLESSIS

In the case of applications in the name of a Company, Partnership or Firm, give full names of signatory/signatories, delete paragraph 1, and enter capacity of each signatory in paragraph 2.
If the applicant is a natural person, delete paragraph 2.
If the right to apply is not by virtue of an assignment from the inventor(s), delete "an assignment from the inventor(s)" and give details of acquisition of right.
For non-convention applications delete paragraph 5.

2003/1344

D M KISCH INC
Patent, Trade Mark &
Copyright Attorneys

South Africa
Local Inventors

ASSIGNMENT OF INVENTION

WHEREAS, I

DU PLESSIS, Tjaart Andries
of
298 Stokkiesdraai
Erasmusrand
PRETORIA

am the inventor of an invention entitled: "PREPARATION OF AN OSTEOINDUCTIVE AGENT"

AND WHEREAS

GAMMATRON (PTY) LTD
of
298 Stokkiesdraai
Erasmusrand
PRETORIA

has for good and sufficient consideration acquired the said invention from me for all countries;

NOW THEREFORE this Deed witnesses that I have assigned the invention to **GAMMATRON (PTY) LTD** its successors, assigns or legal representatives, for all countries, together with the right to apply for Letters Patent in respect thereof in its own name, the assignment taking effect on or before ...18. February..2003...

I agree that when requested, I will without charge to the assignee but at its expense sign all papers, take all rightful oaths, and do all acts which may be necessary, desirable or convenient for securing and maintaining patents for said invention in any and all countries and for vesting title thereto in said assignee, its successors, assigns, or legal representatives.

SIGNED AT PRETORIA THIS 18th DAY OF FEBRUARY 2003.

.....
DU PLESSIS, Tjaart Andries

D. M. Kisch Inc, Sandton

Form P.6

REPUBLIC OF SOUTH AFRICA

PATENTS ACT, 1978

PROVISIONAL SPECIFICATION

(Section 30 (1) - Regulation 27)

OFFICIAL APPLICATION NO.	LODGING DATE	DMK REFERENCE
21 01 2003/1344	22 ~~19 February 2003~~ Post dated 19.05.03	P26152ZA00

FULL NAME(S) OF APPLICANT(S)	
71	GAMMATRON (PTY) LTD

FULL NAME(S) OF INVENTOR(S)	
72	DU PLESSIS, Tjaart Andries

TITLE OF INVENTION	
54	PREPARATION OF AN OSTEOINDUCTIVE AGENT

PREPARATION OF AN OSTEOINDUCTIVE AGENT

INTRODUCTION AND BACKGROUND TO THE INVENTION

This invention relates to a method for the preparation of an osteoinductive agent, the use of such an agent, and to an osteoinductive kit including such agent. This invention further relates to the use of the said kit in the dispensing of such an osteoinductive agent.

It is known to use demineralised bone (DMB) in a biopolymer carrier such as hyaloronic acid (HA), as an osteoinductive agent in reconstructive bone surgery. A first disadvantage of this osteoinductive system is that it requires both the DMB and the associated biopolymer carrier to be prepared under aseptic conditions and dispensed from a customised hypodermic syringe to ensure the sterile presentation of the osteoinductive agent during the surgical procedure.

Further disadvantages of the known osteoinductive system are that it is dispensed as a wet putty in a modified hypodermic syringe and it has to be radiation sterilised and stored at -40°C to prevent any biological or radiation breakdown of the system. It is therefore difficult to store and handle at such low temperatures and the integrity of the system could be jeopardised should the cold chain be broken.

In this specification, the term biopolymer includes within its scope a polymer derived from a biological source, whether plant, microorganism or animal.

OBJECT OF THE INVENTION

It is therefore an object of the present invention to provide a method for the preparation of an osteoinductive agent, the use of such an agent, an osteoinductive kit including such an agent, and to the use of the said kit in the dispensing of such an osteoinductive agent, with which the aforesaid disadvantages can be overcome or at least minimised.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided a method for the preparation of an osteoinductive agent including the steps of:

- modifying a naturally occurring biocompatible biopolymer by subjecting the biopolymer in the solid, or dry state, to a source of ionising radiation in the presence of a mediating gas and annealing the resulting product in the absence of oxygen at a temperature of about 10 to 120°C to render the product in a dry particulate form;
- thereafter removing any residual mediating gas; and
- disposing the product in a hermetically sealed container containing an oxygen-free gas.

The naturally occurring biocompatible biopolymer may comprise collagen. Alternatively the naturally occurring biocompatible biopolymer may comprise demineralised bone (DMB). Further alternatively, the naturally occurring biocompatible biopolymer may comprise a mixture of collagen and DMB.

The DMB and collagen may be subjected to the ionising radiation in the presence of a mediating gas separately from each other and thereafter be mixed. Alternatively, the DMB and collagen may first be mixed and thereafter be subjected to the ionising radiation in the presence of a mediating gas.

The sealed container may be a secondary container and the method may include the further step of disposing the product inside a primary container, which is disposed within the sealed secondary container. The method may include the further step of disposing the sealed secondary container inside a hermetically sealed tertiary container.

The primary container may be in the form of a syringe - type container, having a plunger for dispensing the contents thereof and an opening which is relatively larger than openings of conventional syringes, to allow for the dispensing of relatively viscous contents.

The secondary and tertiary containers may each be in the form of a polymeric pouch.

The space in the primary container not occupied by the product may be filled with the said oxygen-free gas.

The oxygen-free gas may be inert.

The tertiary container may be filled with an oxygen-free gas. This gas may be nitrogen.

The method may include the further step of including another container containing a liquid in the sealed secondary container.

The liquid may comprise pyrogen-free water.

The said hermetically sealed primary-, secondary- and tertiary containers, may be subjected, in kit form, to a terminal radiation sterilisation process.

The method may include the further step of storing the sealed containers and their contents until an osteoinductive agent is required.

The method may include the further step of opening the sealed containers and mixing the sterile liquid with the product in a dry particulate form to hydrate the product to form an osteoinductive agent in the form of a pliable viscous putty.

The method may include the further step of dispensing the osteoinductive agent from the primary container to a bone reconstruction site.

According to a second aspect of the invention there is provided an osteoinductive agent prepared in accordance with the method of the first aspect of the invention.

According to a third aspect of the invention there is provided a kit for preparing and dispensing an osteoinductive agent comprising a modified naturally occurring biocompatible biopolymer which was subjected, in the solid, or dry state, to a source of ionising radiation in the presence of a mediating gas and annealed in the absence of oxygen at a temperature of about 10 to 120°C to render the product in a dry particulate form, the product being disposed in a hermetically sealed container containing an oxygen-free gas.

The naturally occurring biocompatible biopolymer may comprise collagen. Alternatively the naturally occurring biocompatible biopolymer may comprise demineralised bone (DMB). Further alternatively, the naturally occurring biocompatible biopolymer may comprise a mixture of collagen and DMB.

The DMB and collagen may be subjected in the presence of a mediating gas to the ionising radiation separately from each other and thereafter be mixed.

Alternatively, the DMB and collagen may first be mixed and thereafter be subjected to the ionising radiation in the presence of a mediating gas.

The sealed container may be a secondary container and the kit may further include a primary container for containing the product, the primary container being disposed within the sealed secondary container. The kit may include a hermetically sealed tertiary container and the sealed secondary container may be disposed inside the tertiary container.

The primary container may be in the form of a syringe - type container, having a plunger for dispensing the contents thereof and an opening which is relatively larger than the openings of conventional syringes, to allow for the dispensing of relatively viscous contents.

The secondary and tertiary containers may each be in the form of a polymeric pouch.

The space in the primary container not occupied by the product may be filled with the said oxygen-free gas.

The oxygen-free gas may be inert.

The tertiary container may be filled with an inert gas. The inert gas may be nitrogen.

The kit may include another container containing a liquid in the sealed secondary container.

The liquid may comprise pyrogen-free water.

According to a fourth aspect of the invention there is provided a method of reconstructive bone surgery in humans or animals including the steps of:

- providing the kit in accordance with the third aspect of the invention;
- opening the secondary and tertiary containers;
- hydrating the dry particulate product by injecting the sterile liquid into the primary container and mixing the liquid and the product to form putty;
- dispensing the putty into a bone reconstruction site; and
- closing the site to allow bone reconstruction to take place.

Further according to the invention the above steps take place under aseptical conditions.

BRIEF DESCRIPTION OF THE DRAWING

The invention will now be described further by way of a non-limiting example with reference to the accompanying drawing, which is a plan view of a kit for preparing an osteoinductive agent according to a preferred embodiment of the invention.

In accordance with pending US Patent application number 09/805,385 entitled *NEW BIOPOLYMERS BY SOLID STATE IRRADIATION IN AN UNSATURATED GASEOUS ATMOSPHERE*, the radiation crosslinking of DMB results in a 350% increase in the osteoinductive capacity of the DMB and the associated strength of the new bone. Further in accordance with that patent application, the radiation crosslinking of collagen results in a thousand fold increase in the molecular mass of the modified collagen, thus rendering this biopolymer as an excellent carrier for the DMB.

DESCRIPTION OF A PREFERRED EMBODIMENT OF THE INVENTION

Referring to the drawing, a kit according to a preferred embodiment of the invention for preparing an osteoinductive agent, is generally designated by reference numeral 10.

The kit 10 comprises a modified naturally occurring biocompatible biopolymer which was subjected, in the solid, or dry state, to a source of ionising radiation in the presence of a mediating gas and annealed in the absence of oxygen at a

temperature of about 10 to 120°C to render the product in a dry particulate form, the product 12 being disposed in a hermetically sealed secondary container 14 containing an inert oxygen-free gas 16.

The naturally occurring biocompatible biopolymer comprises a mixture of collagen and demineralised bone (DMB). The DMB and collagen mixture is subjected in the presence of a mediating gas to the ionising radiation separately from each other and is thereafter mixed. Alternatively, the DMB and collagen are first mixed and thereafter subjected to the ionising radiation in the presence of the mediating gas.

The kit 10 further includes a primary container 18 for containing the product 12, the primary container being disposed within the sealed secondary container 14. The primary container 12 is in the form of a syringe - type container, having a plunger 18.1 for dispensing the contents thereof and an outlet opening 18.2, which is relatively larger than the openings of conventional syringes, to allow for the dispensing of relatively viscous contents. The opening 18.2 is closed with a removable cap 18.3. A space 18.4 in the primary container not occupied by the product 12 is filled with the said inert gas 16.

The kit 10 further includes a hermetically sealed tertiary container 20 filled with nitrogen gas, the sealed secondary container 14 being disposed inside the

tertiary container 20. The secondary and tertiary containers 14 and 20 are each in the form of a polymeric pouch.

The kit 10 yet further includes another syringe - type container 22 containing a liquid in the form of pyrogen free water 24, the syringe - type container 22 also being disposed inside the sealed secondary container 14. The container 22 is provided with an outlet spout 22.1 which fits inside the outlet opening 18.2 of the primary container 18, to inject the pyrogen-free water 24 into the space 18.4. The opening 22.1 is likewise closed with a cap 22.2.

In use, the secondary and tertiary containers 14 and 20 respectively are opened, the caps 18.3 and 22.2 removed and the water 24 injected into the space 18.4 and mixed with the product 12. The product 12 is thus hydrated to form an osteoinductive putty. The putty is manually dispensed into a bone reconstruction site (not shown) in a human or animal body and the site closed to allow bone reconstruction to take place. It will be appreciated that these steps have to take place under aseptic conditions.

It was found that the crosslinking of collagen results in a carrier for DMB that does not show the undesirable physiological side effects observed with prior art chemically crosslinked alternatives.

In carrying out the method for the preparation of the osteoinductive product 12, the biopolymer must be in the solid state, i.e. dry, in an atmosphere comprising a mediating agent, preferably a low molecular weight unsaturated alkenic or alkynic gas such as ethylene, propylene or acetylene, preferable acetylene. Before introducing the mediating gas to the reaction site, the site must be flushed to remove any oxygen therefrom. All the mediating gas is removed after completion of the process and therefore, the resulting product does not contain any of the mediating gas.

The biopolymer (or the finished or partially finished product 12 made therefrom) from which the active atmosphere has been removed is then saturated with the mediating gas at atmospheric pressure and exposed to a source of ionising radiation which may be either a radioactive isotope such as ^{60}Co (γ-rays) or radiation generated by a high energy (250 keV to 10 MeV) electron accelerator or X-rays generated by the accelerator or any other suitable device.

The minimum absorbed radiation dose may vary from 1 kGy to 50 kGy, depending on the structure of the biopolymer, whether branched or long-chain nature of the product desired, whether of increased molecular weight to form a readily water soluble product or to form either a gel or a membrane product.

For example, the dry DMB and collagen are pre-mixed in the required ratio (2:1) and placed in the primary container 18. This dry mixture of the DMB and

the collagen is then subsequently radiation crosslinked according to the method described in US Patent application 09/805,385 at the optimum minimum absorbed irradiation dose of 16 kGy which is the same for both biopolymers.

Following the irradiation step in the presence of the gaseous mediating agent, and in order to remove any activated species produced by the radiation system, the resulting biopolymer system or new material is subjected to heat treatment (annealing) in the absence of oxygen at elevated temperatures ranging from 40°C to 120°C depending on the heat stability of the biopolymer system which is being modified. This annealing step may ideally be carried out in the presence of the unsaturated gaseous atmosphere or, alternatively, in the presence of an inert gas such as nitrogen or helium, or in a vacuum oven. The former can increase the amount of new product formation, and the latter provides a suitable mechanism for termination of the process.

Following the annealing step, any residual gaseous mediating agent is removed from the modified biopolymeric system by aerating the system, and if necessary, the application of a vacuum process to the treated polymer. This will depend on the retention ability of the material for the gas, which depends on the porosity of the solid system.

Following the annealing step, the crosslinked dry mixture in the primary container 12, together with the syringe - type container 22 containing the

desired volume of pyrogen-free water 24 are sealed hermetically under nitrogen atmosphere in the secondary container 14 that will prevent the ingress of any oxygen. The secondary container 14 is then inserted into the tertiary container 20 and hermetically sealed, followed by the radiation sterilisation of the packaged products – inclusive of the pyrogen-free water.

This method of preparing the crosslinked osteoinductive agent in accordance with the invention has *inter alia* the following advantages:

- The dry osteoinductive product 12 will have a substantially indefinite shelf life, as the osteoinductive putty is prepared freshly directly before use. The current need for cold storage of such osteoinductive agents is thus obviated.

- Because of the blanketing of the components with nitrogen prior to radiation sterilisation and storage, virtually no radiation-induced oxidative degradation of the dry osteoinductive mixture or the syringes takes place. This results in the enhanced shelf life of the product.

- This technique allows the osteoinductive mixture and water for re-hydration to be subjected to a terminal radiation sterilisation process

and the associated very high degree of sterility assurance and safety to the patient.

It will be appreciated that variations in detail are possible with the use and preparation of an osteoinductive agent, with an osteoinductive kit including such agent and with the use of the said kit in the dispensing of such an osteoinductive agent, according to the invention without departing from the scope of this disclosure.

DATED THIS 19. DAY OF FEBRUARY 2003.

D M KISCH INC

PATENT ATTORNEYS FOR THE APPLICANT

GAMMATRON (PTY) LTD

2003/1344

ONE SHEET

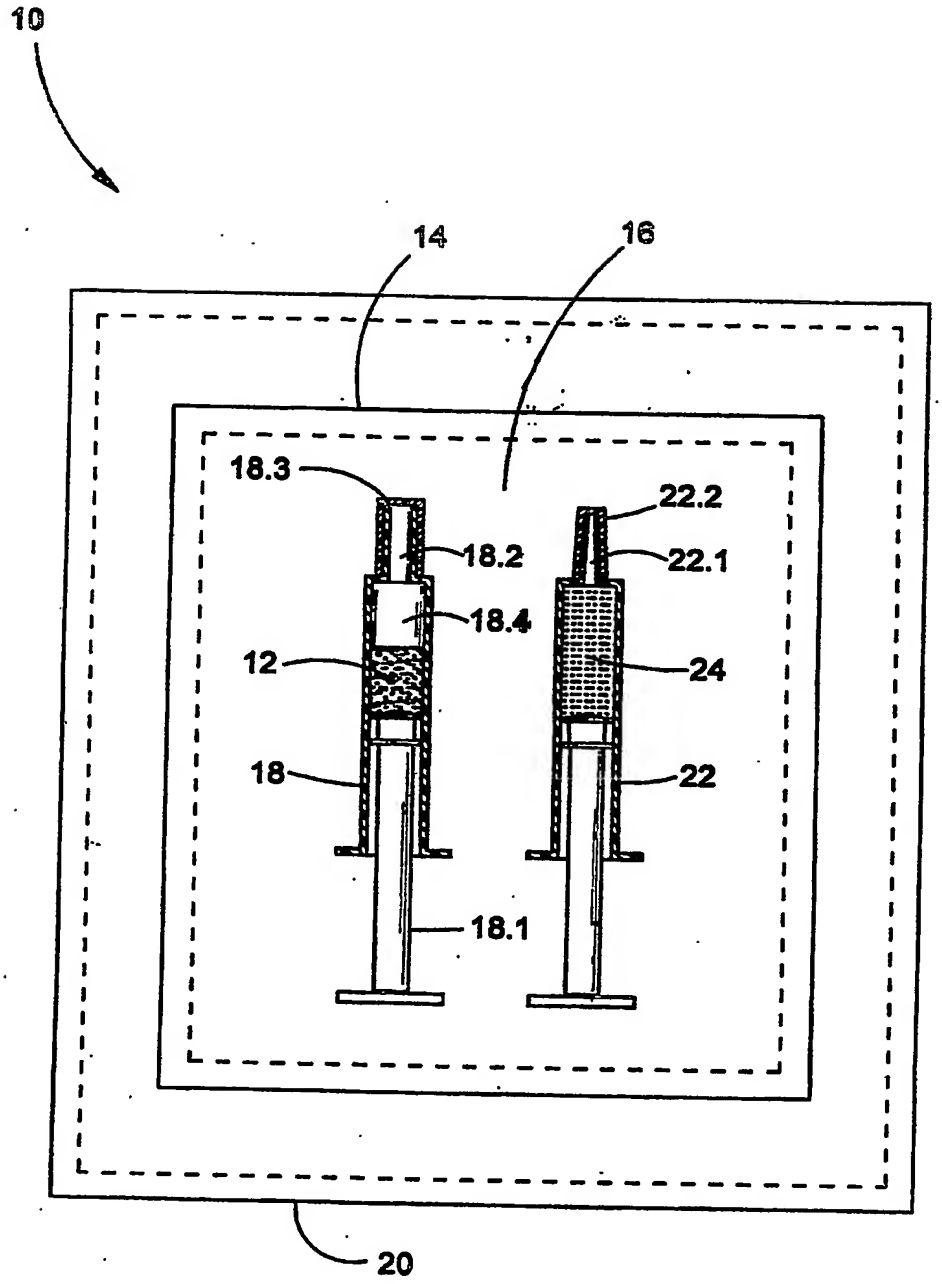

FIGURE 1

D.M. KISCH INC.
Patent Attorneys
JOHANNESBURG

Patent Attorney for the Applicant

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